American Journal of Hematology 58:95–99 (1998)

Novel Cytomorphology of the Giant Proerythroblasts of Parvovirus B19 Infection

Prasad Rao Koduri*

Division of Hematology, Cook County Hospital, Chicago, Illinois
Rush Medical College, Chicago, Illinois

The morphology of the giant proerythroblasts (GPE) in air-dried and Wright-Giemsa-stained smears of bone marrow in 16 patients with pure red cell aplasia (PRCA) caused by parvovirus B19 infection is described. B19 infection was diagnosed by the presence of the virus or viral DNA and/or IgM antibodies. Twelve patients had chronic hemolytic anemia and aplastic crisis and 4 patients had AIDS with chronic PRCA. In patients with chronic hemolytic anemia and aplastic crisis, GPE were not detectable in bone marrow biopsies that showed any degree of recovery of erythropoiesis. The GPE morphology was quite variable. The early (basophilic) GPE measured 25 to 35 μm in diameter, had a narrow rim of intensely blue and often vacuolated cytoplasm with pseudopodia, round nuclei with compact uncondensed chromatin, and an indistinct and inclusion-like purple-colored tinctorial change. The ''intermediate'' and ''late'' GPE measured 25 to 45 μm in diameter and showed cytoplasmic swelling, gradual loss of cytoplasmic basophilia, and fraying of the cytoplasm with focal rupture; the nuclei showed an increase in volume, a highly uncondensed and coarse sieve-like chromatin, and 1 to 3 prominent, pale to moderate purple inclusion-like nucleoli or inclusions. Bare nuclei similar in size and chromatin pattern to those of the GPE were present in proximity to the GPE and may have arisen from the GPE by dissolution of the cytoplasm. The glassy intranuclear inclusions with central clearing, the so-called lantern cells described in formalin-fixed tissues of patients with B19 infection, were absent in all cases. These findings suggest that direct toxic cell injury rather than apoptosis may be involved in the pathogenesis of erythroid aplasia in B19 infection. Am. J. Hematol. 58:95–99, 1998. © 1998 Wiley-Liss, Inc.

Key words: red cell aplasia; erythroblasts; bone marrow examination; parovirus B19; aplastic crisis

INTRODUCTION

Parvovirus B19 is a single-stranded DNA virus that causes transient red cell aplasia with aplastic crisis in patients with chronic hemolytic anemia and chronic pure red cell aplasia in patients with immunodeficiency [1–3]. Taken together, pure red cell aplasia (PRCA) in smears of bone marrow aspirate and the presence of characteristic giant proerythroblasts (GPE) are pathognomonic of B19 infection [4–6]. In air-dried and Wright-stained smears of bone marrow, the GPE have been described as large cells with dark blue, often vacuolated cytoplasm, fine megaloblastoid chromatin, and cytoplasmic pseudopodia [3,7–9]. However, systematic studies of the cytomorphology of GPE in clinical specimens of bone marrow aspirate have not been reported. Examination of bone marrow smears of patients with PRCA due to B19 infection showed that there is considerable variation in the morphology of GPE that has not been describing previously. The following report describes the morphologic appearances of GPE in bone marrow smears in 16 patients with PRCA due to B19 infection.

PATIENTS AND METHODS

The records of all patients diagnosed with (1) chronic hemolytic anemia and aplastic crisis and (2) AIDS and persistent red cell aplasia caused by parvovirus B19 in the Division of Hematology at Cook County Hospital during the years 1981–1988 and 1992–1996 were reviewed. All patients were seen in consultation by the author. Complete blood counts, reticulocyte counts, se-

*Correspondence to: Prasad Rao Koduri, M.D., Division of Hematology, Hektoen Bldg, Room 817, 627 S. Wood Street, Chicago, IL 60612.

Received for publication 30 April 1997; Accepted 12 November 1997

rum iron, total iron binding capacity, and serum vitamin B12 and folate levels were determined in all patients. The diagnosis of B19 infection in the patients seen prior to 1988 was by methods previously described [10] and required the demonstration of the virus and/or IgM antibody. Since 1992, the presence of IgM and IgG antibodies to B19 was determined by an indirect immunofluorescence test using a commercial kit (Incstar Corporation, Stillwater, MN), and B19 DNA was detected by PCR (Speciality Laboratories, Santa Monica, CA). The bone marrow smears of these patients were reviewed, paying particular attention to the number and morphology of GPE. The diameter of GPE was determined with the help of a stage micrometer (Leica Inc., Deerfield, IL). Photomicrographic slides of the micrometer scale were taken using the 40× and 100× objectives and Kodak® Ektachrome 64T film. These slides were then used to make measurements of the sizes of the GPE on photomicrographic slides of the same magnification.

RESULTS

Patients were divided into in two groups: those with chronic hemolytic anemia and those with AIDS.

Chronic Hemolytic Anemia

During the study period, 21 patients with chronic hemolytic anemia were diagnosed with aplastic crisis caused by B19. Of these, 19 patients had sickle cell anemia (SS), one patient had S-C hemoglobin disease, and another had beta-thalassemia intermedia. Some of these patients have been reported previously [10–13]. All of the patients were febrile and had symptoms of a viral syndrome at admission. In patients with sickle cell anemia, the hemoglobin values ranged from 2.3 to 5.9 g/dL, and the values were 8.0 and 8.4 g/dL, respectively, in the patients with thalassemia intermedia and S-C hemoglobin disease. In all patients, the reticulocytes were <0.1%.

In 7 cases, the bone marrow showed variable degrees of recovery of normal erythropoiesis. GPE were not present in the bone marrow smears in any of these seven cases. A bone marrow biopsy was not done in one patient. GPE were demonstrable in the bone marrow smears of all remaining 13 patients with hemolytic disease and PRCA; one patient in whom the acute serum sample was not available for virological studies was excluded from further analysis.

AIDS

A total of 9 patients suffering from AIDS were consulted for severe anemia (Hb <5 g/dL) and reticulocytopenia (reticulocytes <0.1%) and were strongly suspected of having B19 infection. All patients in this group were afebrile and had non-specific symptoms referable to the severe anemia. In two patients, the bone marrow biopsy showed PRCA and characteristic GPE but a test for B19 DNA was not done. In three other patients, the serum or bone marrow tested positive for B19 DNA but bone marrow biopsy showed evidence of an early recovery of erythropoiesis and no GPE. The remaining four patients had PRCA and GPE on bone marrow biopsy, and tested positive for B19 DNA and are included in this study. One of these patients has been reported previously [14]. The serum levels of folate and vitamin B12 in these patients were normal.

There was a difference in the morphological appearance of the bone marrow of patients suffering from chronic hemolytic anemia and those suffering from AIDS. In those patients with hemolytic disease, the bone marrow was hypercellular with a marked increase in the megakaryocytes in all patients. In 7 of 21 patients in this group, there was an increase in small round lymphocytes in the bone marrow smears. In contrast, the bone marrow cellularity was normal in the group of patients with AIDS and there were no instances of an increase in bone marrow lymphocytes.

The morphology of the GPE was similar in both groups of patients and is illustrated in Figures 1–3. The GPE measured from 25 to 45 μm in diameter and showed a spectrum of morphological changes. For ease of description they are organized into early (basophilic) GPE, intermediate, and late forms.

Early (Basophilic) GPE (Fig. 1A,B)

These cells had a high nucleo-cytoplasmic ratio and a well-demarcated narrow rim of intense coarse blue cytoplasm. There were up to 10 or more sharply defined pin-head-sized vacuoles in the cytoplasm. The cell size varied from 25 to 32 μm. Many of these ''basophilic'' GPE showed small broad-based cytoplasmic ''pseudopodia'' or so-called ''dog-ear'' projections [9], which were budding from the cell (Fig. 1B). Some of these cytoplasmic ''buds'' were observed as free lying structures in close proximity to the GPE. The nuclei of GPE were round and had fine, uncondensed, uniformly dispersed compact chromatin. Intranuclear inclusions were not readily apparent in the basophilic GPE. Some basophilic GPE showed an indistinct and irregular purple staining area in the nucleus with no distinct limiting membrane, which I have termed an ''inclusion-like change.''

Intermediate GPE (Fig. 2A)

These cells were generally larger in size than the early or basophilic GPE and measured 45 μm in their maximum diameter. The intermediate GPE showed a spectrum of morphologic appearances consisting of variable loss of cytoplasmic basophilia concomitant with an apparent increase in the amount of cytoplasm and nuclear volume. Occasional intermediate GPE showed asynchronous hemoglobinization of the cytoplasm similar to that described in megaloblasts. The nuclei of the intermediate

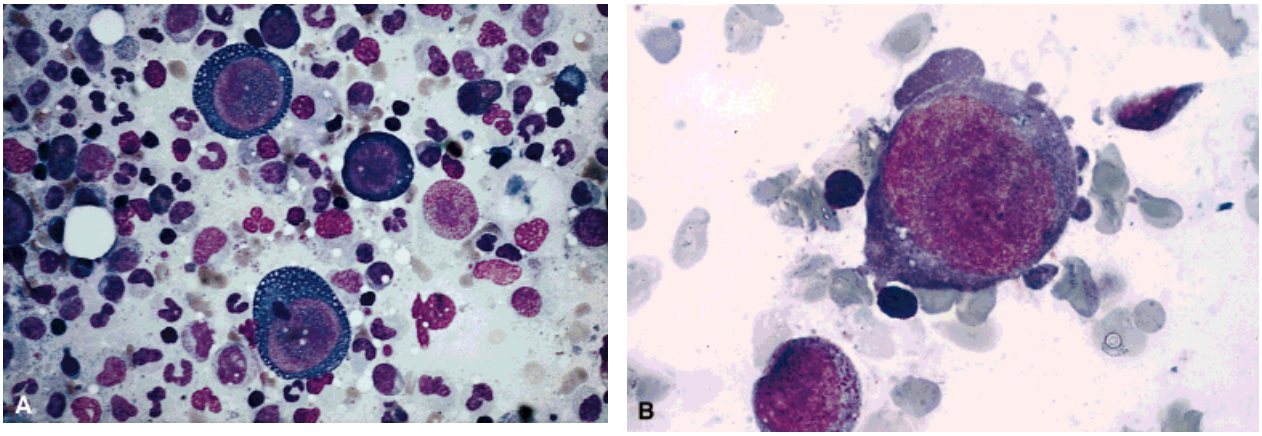

Fig. 1. A: Basophilic giant proerythroblasts (GPE) with intensely blue, vacuolated cytoplasm and an indistinct purple-colored inclusion-like change in the nucleus. Also seen is a bare nucleus in the center right field. (original magnification, ×400). B: A basophilic GPE with a rim of basophilic cytoplasm with pseudopodia or ''dog ears.'' The nucleus has a compact uncondensed chromatin pattern (original magnification, ×1,000).

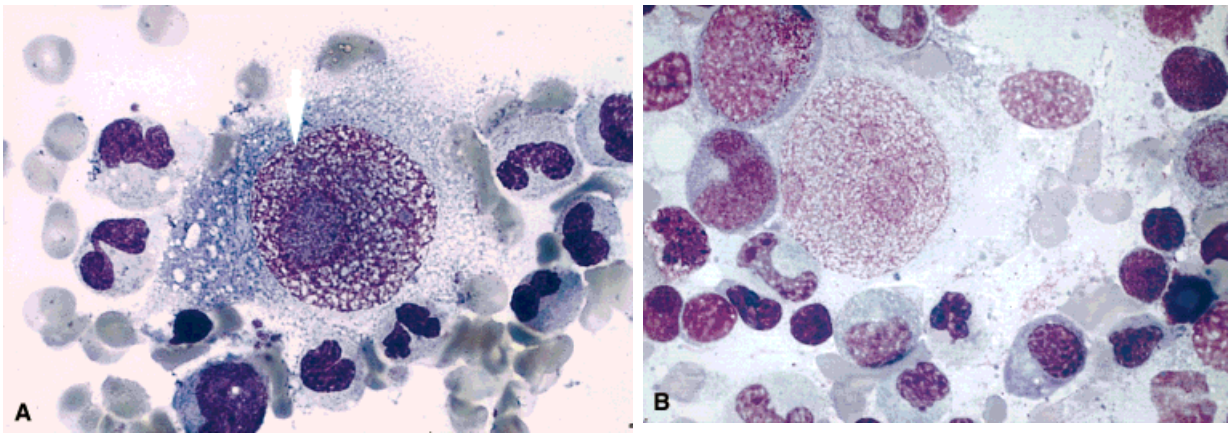

Fig. 2. A: Intermediate GPE. Coarse, highly uncondensed chromatin with a nucleolus or inclusion, and a decrease in cytoplasmic basophilia (original magnification, ×1,000). B: Late GPE with pale gray-blue, frayed cytoplasm and a coarse, highly uncondensed chromatin (original magnification, ×1,000).

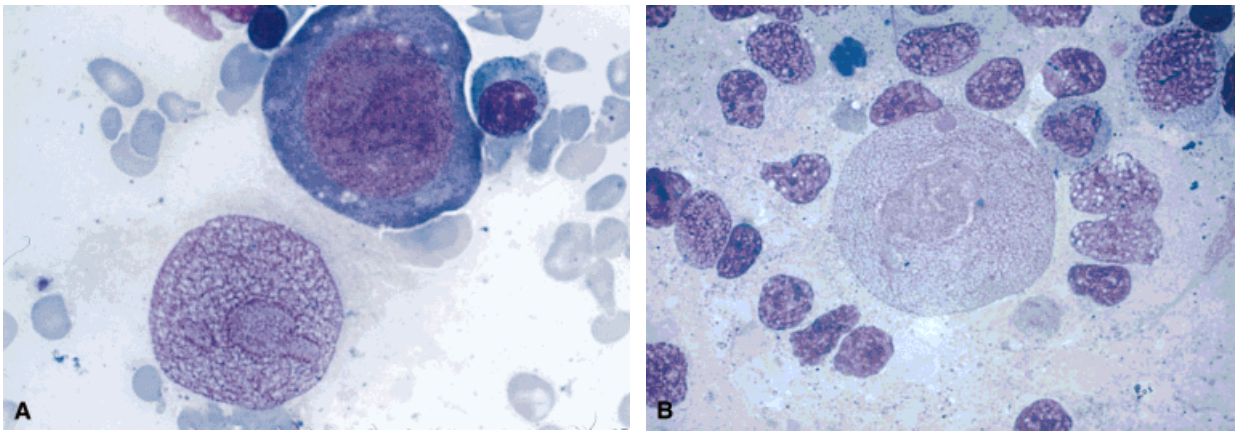

Fig. 3. A: A bare nucleus with a coarse, highly uncondensed chromatin and a pale purple-colored intranuclear nucleolus or inclusion (original magnification, ×1,000). A basophilic GPE is also seen. B: Bare nucleus with uncondensed sieve-like chromatin and a pale purple-colored nucleolus or inclusion (original magnification, ×1,000).

GPE had highly uncondensed coarse sieve-like chromatin with individual chromatin strands often showing a beaded appearance. Many of these cells showed 1 to 3 prominent pale to moderate purple nucleoli or inclusions. There was no condensation of heterochromatin along the periphery of the nucleus of the GPE as seen in histologic sections of formalin-fixed tissues stained with hematoxylin-eosin and in electron microscopic studies [15–20].

Late GPE (Fig. 2B)

These cells had nuclei that were often slightly larger in diameter than those of the basophilic GPE. The cytoplasm was a pale gray-blue to nearly water clear and showed fraying of the margins in focal areas. Occasional GPE showed a partial extrusion of the nucleus at one pole through what appeared to be a rupture in the cell margin. The nuclei had coarse mesh-like chromatin and one to two prominent pale to moderate purple nucleoli or inclusions.

Bare Nuclei (Figs. 1 and 3A,B)

Bare nuclei were often seen in close proximity to the GPE and were generally similar in size to the nuclei of late GPE. However, they had no discernible cytoplasm. Their coarse meshwork of chromatin resembled that of the late GPE and had one to two prominent pale to moderate purple-colored inclusions or nucleoli. In some cases, 3 to 6 bare nuclei could be seen in a high-power field (50× objective).

GPE were not seen in bone marrow biopsies of patients with chronic hemolytic anemia who had any degree of recovery of normal erythropoiesis. A few normal proerythroblasts could be seen together with GPE in the bone marrow smears of some patients with AIDS; late erythroid precursors were, however, virtually absent in all cases. In most cases, the entire spectrum of morphologic appearances of the GPE could be seen in a single bone marrow smear.

DISCUSSION

GPE are the hallmark of B19 infection. GPE were first recognized by Owren in 1948 in the bone marrow of patients with aplastic crisis [21]. Before the discovery of the virus, cells strikingly similar to the GPE of B19 infection had been described or illustrated by earlier authors in clinical reports of transient erythroid aplasia [22–24].

The size, chromatin structure, staining characteristics, and proximity to the GPE suggest that the bare nuclei may be derived from the basophilic GPE. This hypothesis is supported by the presence of GPE with a spectrum of morphologic changes intermediate between the basophilic GPE and the bare nuclei. The swelling of the cytoplasm, loss of basophilia, and ultimate dissolution and rupture of the cytoplasm may be an indication of the cells' inability to synthesize new protein and maintain ionic and water balance.

Some similarities between the GPE of B19 infection and megaloblasts are apparent. These include the large size, the fine ''megaloblastoid'' chromatin [7], and the asynchronous hemoglobinization that is seen in some later forms of the GPE. However, while hemoglobin synthesis appears to progress fairly well in megaloblasts, there appears to be a lack of hemoglobinization in most GPE [25].

Air-dried smears of bone marrow stained with Wright-Giemsa stain may not contain cells with readily recognizable inclusions [26]. Formalin fixation for 15 min of air-dried smears of bone marrow has been reported to improve the detection of the characteristic intranuclear inclusions [26]. Thus, it is possible that the intranuclear inclusions with central clearing seen by light microscopy and demonstrated in formalin-fixed tissues [15–18] may be a fixation ''artifact'' [26]. The glassy intranuclear inclusions with central clearing, the so-called lantern cells [16,17,19,20], were not observed in any of the cases described in the present study.

The mechanism of erythroid aplasia in B19 infection is not fully understood. Based on the peripheral condensation of chromatin and the formation of cytoplasmic blebs, apoptosis has been inferred [27]. However, the light microscopic observations of air-dried, non-formalin fixed smears of bone marrow in our patients suggest swelling and disruption of the cytoplasm with no signs of chromatin condensation or margination in the nucleus. These findings are indicative of cell lysis by toxic injury, probably as a result of a direct cytopathogenic ''lytic'' effect of the virus on erythroid progenitor cells. The gene encoding the non-structural protein (NS1) of B19 has been shown to be lethal in transfected cells [28] and its cytotoxic effects may be responsible for the red cell aplasia in B19 infection [3]. This view is supported by the morphologic changes of the erythroid precursor cells observed in our patients.

REFERENCES

1. Pattison JR, Jones SE, Hodgson J, Davis LR, White JM, Stroud CE: Parvovirus infections and hypoplastic crisis in sickle cell anaemia. Lancet 1:664–665, 1981.
2. Frickhofen N, Abkowitz JL, Safford M, Berry M, Antunez-de-Mayolo J, Astrow A, Cohen R, Halperin I, King L, Mintzer D, Cohen B, Young NS: Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): A treatable cause of anemia in AIDS. Ann Int Med 113:926–933, 1990.
3. Young NS: Parvoviruses. In: Fields BN, Knipe DM, Howley PM (eds): ''Fields Virology,'' 3rd ed. Philadelphia: Lippincott-Raven, 1996, pp 2199–2220.
4. Morey AL, Fleming KA: Immunophenotyping of fetal haemopoietic cells permissive for human parvovirus B19 replication in vitro. Br J Haematol 82:302–309, 1992.
5. Mitsuyasu RT, Lambertus M, Goetz MB: Transfusion-dependent ane-

mia in a patient with AIDS (Infectious Disease Rounds). Clin Infect Dis 15:533–539, 1992.
6. Carper E, Kurtzman GJ: Human parvovirus B19 infection. Curr Opin Hematol 3:111–117, 1996.
7. Frickhofen N, Chen ZJ, Young NS, Cohen BJ, Heimpel H, Abkowitz JL: Parvovirus B19 as a cause of acquired chronic pure red cell aplasia. Br J Haematol 87:818–824, 1994.
8. Ozawa K, Kurtzman G, Young N: Productive infection by B19 parvovirus of human erythroid bone marrow cells in vitro. Blood 70:384–391, 1987.
9. Harris JW: Parvovirus B19 for the hematologist. Am J Hematol 39: 119–130, 1992.
10. Rao KRP, Patel AR, Anderson MJ, Hodgson J, Jones SE, Pattison JR: Infection with parvovirus-like virus and aplastic crisis in chronic hemolytic anemia. Ann Int Med 98:930–932, 1983.
11. Koduri PR, Patel AR, Pinar H: Acute hepatic sequestration caused by parvovirus B19 infection in a patient with sickle cell anemia. Am J Hematol 47:250–251, 1994.
12. Koduri PR, Naides SJ: Aseptic meningitis caused by parvovirus B19. Clin Infect Dis 21:1053, 1995.
13. Koduri PR, Naides SJ: Transient blood plasmacytosis in parvovirus B19 infection: A report of two cases. Ann Hematol 72:49–51, 1996.
14. Koduri PR, Kumapley R, Khokha ND, Patel AR: Red cell aplasia caused by parvovirus B19 in AIDS: Use of i.v. immunoglobulin. Ann Hematol 75:67–68, 1997.
15. Young N, Harrison M, Moore J, Mortimer P, Humphries RK: Direct demonstration of the human parvovirus in erythroid progenitor cells infected in vitro. J Clin Invest 74:2024–2032, 1984.
16. Burton PA: Intranuclear inclusions in marrow of hydropic fetus due to parvovirus infection. Lancet 1:1155, 1986.
17. Anand A, Gray ES, Brown T, Clewley JP, Cohen JB: Human parvovirus infection in pregnancy and hydrops fetalis. N Engl J Med 316: 183–186, 1987.
18. Sosa CE, Mahony JB, Luinstra KE, Sternbach M, Chernesky MA: Replication and cytopathology of human parvovirus B19 in human umbilical cord blood erythroid progenitor cells. J Med Virol 36:125–130, 1992.
19. Schwarz TF, Nerlich A, Hottentrager B, Jager G, Wiest I, Kantimm S, Roggendorf H, Schultz M, Gloning K-P, Schramm T, Holzgreve W, Roggendorf M: Parvovirus B19 infection of the fetus. Am J Clin Pathol 96:121–126, 1991.
20. Pilavdzic D, Lines LD, Petric M, Silver MM: Immunoelectron microscopic identification of human parvovirus B19. Ultrastruct Pathol 18: 417–422, 1994.
21. Owren PA: Congenital hemolytic jaundice. The pathogenesis of the ''hemolytic crisis.'' Blood 3:231–248, 1948.
22. Dameshek W, Bloom ML: The events in the hemolytic crisis of hereditary spherocytosis, with particular reference to the reticulocytopenia, pancytopenia and an abnormal splenic mechanism. Blood 3:1381–1410.
23. Kho L-K: Erythroblastopenia with giant pro-erythroblasts in kwashiorkar. Blood 12:171–182, 1957.
24. Chanarin I, Barkhan P, Peacock M, Stamp TCB: Acute arrest of haemopoiesis. Br J Haematol 10:43–49, 1964.
25. Ozawa K, Kurtzman G, Young N: Productive infection by B19 parvovirus of human erythroid bone marrow cells in vitro. Blood 70:384–391, 1987.
26. Krause JR, Penchansky L, Knisely AS: Morphological diagnosis of parvovirus B19 infection. A cytopathic effect easily recognized in air-dried, formalin-fixed bone marrow smears stained with hematoxylin-eosin or Wright-Giemsa. Arch Pathol Lab Med 116:178–180, 1992.
27. Morey AL, Ferguson DJP, Fleming KA: Ultrastructural features of fetal erythroid precursors infected with parvovirus B19 in vitro: Evidence of cell death by apoptosis. J Pathol 169:213–220, 1993.
28. Ozawa K, Ayub J, Kajigaya S, Shimada T, Young N: The gene encoding the nonstructural protein of B19 (Human) parvovirus may be lethal in transfected cells. J Virol 62:2884–2889, 1988.